Increased density of Tregs in Oral Lichen Planus

By: Åshild Høva

Supervisor: Trond S. Halstensen, Institute of Oral Biology.

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ABSTRACT

Background: T cell mediated autoimmunity is considered to be involved in the pathogenesis of OLP. Studies demonstrate that Foxp3 transcription is significantly elevated in OLP lesions comparing with controls. This directly suggests that Foxp3+ Treg cells may participate in the pathogenesis of OLP. The crucial roles of the CD4+Foxp3+CD25+ Treg cells have been identified in a series of autoimmune and/or inflammatory diseases. Its role in the pathogenesis of OLP should not be overlooked. Sparse data are available on Foxp3+ Treg cells in OLP

Aim: The aim of this study was to investigate whether some of the T cells in the infiltrate of OLP are of a regulatory type.

Method: By the use of labeled antibodies as specific reagents through antigen-antibody interactions immunhistochemistry was used to localize antigens in tissue sections from biopsies from twelve different patients. In this study multicolor-fluorescence was used to examine CD3, CD25, CD152 and Foxp3.

Results: A fraction of median 30 % of the subepithelial T cells coexpressed Foxp3. The majority of these (median 55%, range 15%-69%) were CD25+ Treg cells. The amount of CD152+ Treg cells among CD3+Foxp3+ cells was median 31% (range 5%-67%). In addition, the exact same markers are counted for in biopsies from five patients in which the histopathological picture did not correlate with OLP/LR. The result from the histopathological analysis here did not variate signigicantly from the above described results.

Conclusion: The results from this study are compared with, and meant as a compliment to the study of Koren *et al*. Both studys demonstrate an increased number of Treg cells compared with a control-gruop. Immunhistochemical analysis of a group of biopsies from inflammatory lesions not diagnosed OLP/LR may raise a question about the diagnostic criteriae of OLP/LR, and may also demonstrate that the Treg cell is an important target when it comes to treatment of other inflammatory disorders as well.

ABBREVATIONS

OLP	Oral lichen planus
LR	Lichenoid reaction
T cell	T lymphocyte
Treg	T regulatory cell
Th1	T helper cells 1
Th2	T helper cells 2
Th17	T helper cells 17
IL-17	Interleukin-17
IL-2	Interleukin-2
IL-2R	IL-2 receptor
Teff cell	T effector cell
APC	Antigen precenting cell

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First of all I would like to thank Professor Trond Sudby Halstensen, head of Laboratory of immunology for never being far away with his extreme enthusiasm and expertise on this area. Workday, holyday or when ill on his sofa, he was allways only a phonecall away for eager discussions and a helpful advice. I am very greatful for getting unlimited axcess to his lab and very expensive equipment. Whithout this generosity, knowledge and availability I would never have been able to conduct my study. I would also like to thank the Faculty of Dentistry at the University of Oslo for assignment of a student summer grant which made me able to devote the summer 2011 to this project.

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I am greatfull for Magaret Korens previous work in this project. It has been my guiding star and my fundament. My work is meant as a compliment to her study on regulatory T cells in OLP.

Oslo, May 6, 2012

INTRODUCTIONS

OLP is a common mucocutanous inflammatory disorder that occurs at sites of stratified squamous epithelia. OLP affects 1-3 % of the population, with notable variation by geograpy and diagnostic criteria. Epidemiological studies are hampered by the lack of clear diagnostic criteria; varied clinical presentation; and the fact that the most common form of OLP, reticular, is asymptomatic and therefore underdiagnosed. Women are affected more commonly than men. Onset of disease occurs between 30 and 60 years of age (1).

OLP is classified morphologically into six subgroups; reticular, white, erythematosus, atrophic, bullous and ulcerated/erosive (1). The three latter are assosciatet with discomfort while the three former often are asymtomatic (2). Multiple morphologies may be present simultaneously. The predominant clinical morphology may change over time with more severe forms occuring in older patients (1). The early lesion often consists of white papules and takes various shapes over time (2). OLP characteristically presents with multiple lesions in a bilateral and roughly symmetric distribution. OLP most commonly involves the buccal mucosa, gingiva, dorsum of the tongue, labial mucosa, and lower vermilion lip. Patients who present with isolated lesions develop multiple sites of involvment over time (1). OLP can undergo malign transformation. Screening for changes in oral mucosa are therefore recomended. Dentists do have an unice posision in relation to detect oral cancer (2).

The etiology of OLP is unknown. However, OLP appears to be a T cell mediated autoimmune disease. Precipitating factors may include trauma, stress and infectious agents. The peripheral immune suppressor function is altered in OLP, and the balance between help and suppression by T cells may determine disease activity (1).

Today, a lot of research is done to map Treg cell subgroups, their underlying mechanisms including induction of Treg cells and direct suppression of T cell responses. This is an important basis for understanding the regulation of autoimmune diseases. Treg cells and dendrittic cells play an important role in the pathogenesis of OLP.

Research on these cells will lead us closer to an understanding of the mechanisms behind this disease, and closer to the ability to offer functional treatment in this division.

Treg cells are a subgroup of CD4+ T cells that protect us against autoimmune disease and prevent T cells from reacting to autoantigens by transforming them into induced Treg cells (iTreg). In addition there are natural Treg cells (nTregs) that are created in thymus.

This project seeks to investigate the immunological picture in OLP. The superior goal here is to find out which cells are represented in the subepithelial infiltrate of OLP with a distinct view to Treg cells and their specific markers. Cand.odont Margaret Koren has earlier shown that allmost 50 % of all T cells in the OLP infiltrate are Treg cells as described above. Here, Korens work is extended, partly by concluding Korens analysis. The patient matherial here is enlargend in co-operation with dr.odont. Bente Brokstad Herlofsen, IKO.

BACKGROUND AND THEORY

T-lymphocytes

T cells or T-lymphocytes play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the prescence of a T cell-receptor (TCR) on the cell surface. They are called T cells because they mature in thymus. There are several subsets of T cells, each with a distinct function (3).

Treg cells

Regulatory T cells (Treg) are together with T helper-cells (Th-cells) that secrete IL-17 newly described T cell subsets that have raised fundamental questions about lineage commitment and fate determination of CD4+ T cells. Classically CD4+ T-helper (Th) cells have been considered to belong to one of two subsets – Th1 cells and Th2 cells – each of wich has unique cytokin products, signalling pathways and lineage-specific transcription factors or master regulators. Now there is four such subsets; Tregs, Th17, Th1 and Th2 **(Figure 1)** (4).

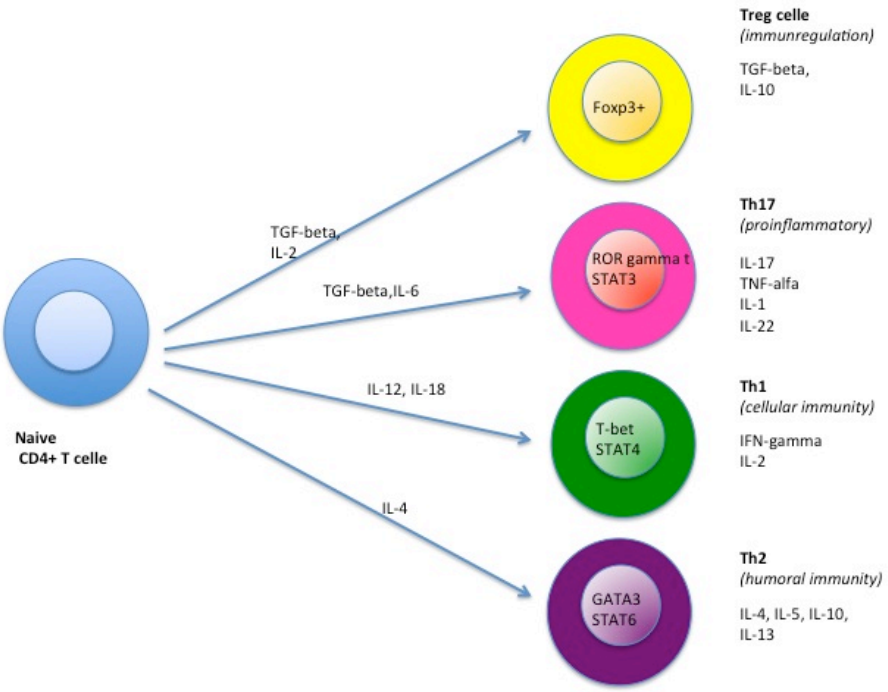

Figure 1: *Schematic representation of the differentiation of naive CD4 T cells (9).*

Among the various linages of effector T cells there are multiple subsets of regulatory T cells including natural CD4+FOXP3+CD25+ regulatory T cells (nTreg) that develope and emigrate from the thymus to performe their role in immune homeostasis. Induced Tregs (iTreg) are non regulatory CD4+ T cells that must be stimulated to gain suppressor function. nTreg and iTreg play an important role in suppressive control of both innate and adaptive immunity, *in vivo* (11)(5). nTreg and iTreg cells originate in response to transforming growth factor(TGF)-beta. nTreg develope from thymic CD4+ T cell precursors in the precence of TGFbeta and IL-2. iTreg develope from naive CD4+ T cells in high levels of TGF-beta and signalling through STAT5 in the tissue microenvironment. This results in upregulation of the transcription factor Foxp3 *(4)(5).* Wheras IL-6 in addition to TGF-beta promotes differentiation of the naive T helper (Th) cell into IL-17 producing CD4+ T-helper (Th17) cells (10)**(Figure 1)(Figure 2)**.

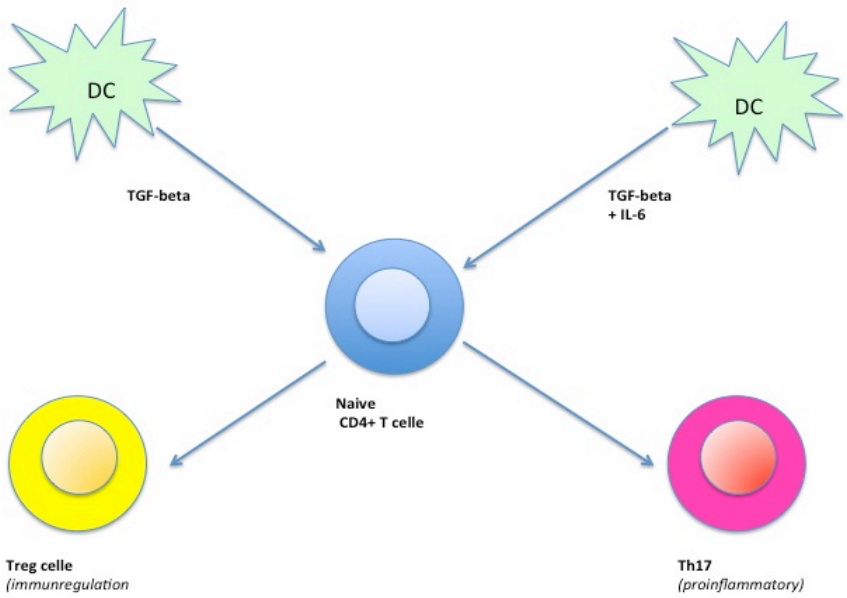

Figure 2: *Differentiation of Naive CD4+ T cells into iTreg cells and Th17 cells*

Resting Tregs from noninflamed tissues exhibit little suppressor activity. Under certain inflammatory conditions and disease processes, such as autoimmunity and cancer (11), resting Tregs may undergo rapid reprogramming to aquire helper/effector functions. The exact role of local inflammation in triggering Treg activation is unknown, but it seems likely that inflammation plays an important role (both as an activator of local

APCs and a driver of the local cytokin milieu) (6). Activation may for example happen through TCR cross-linking *in vitro*. Thus, simply counting the numbers of Foxp3+ Tregs in physiologic settings does not address wether these Tregs are resting or activated. Certain markers appear to correlate with an activated or effector memory phenotype in Tregs. Tregs are defined by CD25, CD39, Foxp3, CD152 and GITR (6), and they produce high levels of IL-10, IL-35 and TGFbeta (4).

Treg cells and autoimmune mediated disease

It is becoming evident that regulation of Th17 cells may play a significant role in the pathogenesis in multipple inflammatory and autoimmune disorders (7). Loss of the balance between Th17 and Treg cells will break immune homeostasis in the host and lead to the development of autoimmune diseases (5).

CD4+CD25+ regulatory T cells (Treg) are a subset of T-lymphocytes that play a central role in inducing and maintaining immunologic tolerance to self and non-self. Most surface proteins that are expressed on CD4+CD25+ Treg cells such as CD152, GITR and PD-L1 can also be found on activated T responder cells. On the other hand, Foxp3, an X chromosome-encoded forkhead transcription factor family member, is indispensable for the developement and function of CD4+CD25+ Tregs, which indicate that Foxp3 is a faithful and specific marker for Tregs. In recent years, abnormalities in number and function of Foxp3-expressing CD4+CD25+ Treg have been identified in a number of inflammatory diseases, including psoriasis, multiple sclerosis, autoimmune polyglandular syndrome type II, rheumatoid artritis, myasthenia gravis and type I diabetes. This evidence indicates that Foxp3-expressing CD4+CD25+ Treg cells may be a target for the development of new treatments of inflammatory/autoimmune diseases in the future (6).

Treg cells in OLP

A large body of evidence support a role of immune dysregulation in the pathogenesis of OLP. Antigen-specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen-specific keratinocyte killing by CD8+ cytotoxic T cells. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase (MMP) activation in OLP lesions. These mechanisms may combine to cause T cell accumultaion in the superficial lamina propria, basement membrane disruption, intra

epitelial T cell migration, and keratinocyte apoptiosis in OLP. Obviously, the abnormality of immunological regulation may contribute to its pathogenesis.

Studies demonstrate that Foxp3 transcription is significantly elevated in OLP lesions comparing with the controls. This directly suggest that Foxp3+ Treg cells may participate in the pathogenesis of OLP.

Studies also indicate that Foxp3 demonstrate a preferential staining of the CD4+CD25+ T cell population. Immunohistochemistry have demonstrated few Foxp3+ cells in tissue sections from normal oral mucosa. However, Foxp3+ T cells are easily detectable in OLP lesions, and mainly dispersed at the lamina propria of lesions. Moreover, the density of Foxp3+ T cells is negative correlation to disease activity in OLP, and the density of Foxp3+ T cells is significantly lower in erythematosus/erosive OLP lesions than in retikular OLP lesions.

As T cell-mediated autoimmunity is considered to be involved in the pathogenesis of OLP, the immune regulatory dysfunction may contribute to development and refractoriness of OLP. Several previous investigations indicated that OLP chronicity may be due, in part, to a defect in the tumor growth factor-beta (TGF-beta)/interleukin-4 (IL-4) immunosuppressive pathway involving insufficient numbers of IL 4-secreting Th2 and TGF-beta-secreting Th3 regulatory T cells. But observations have showed that the expression of IL 4 was upregulated in local lesions of OLP and its protective role seems not to attenuate effectively the excessive immune response mediated by IFN-gamma in OLP. Therefore, there may be other more important mechanisms of immunological regulations contributing to OLP pathogenesis. As mentioned above, the crucial roles of CD4+CD25+Foxp3+ Treg cells have been identified in a series of autoimmune and/or inflammatory diseases, its role in pathogenesis of OLP should not be overlooked. Sparse data are available on Foxp3+ Treg cells in OLP.

Given the fact that Foxp3+ Treg cells have an important role in the machanisms of peripheral immune tolerance and the prevention of pathogenic autoimmunity, investigators have endeavorted to explore the potentials of Foxp3+ Treg cells in the treatment of inflammatory and autoimmune disorders. Many studys have shown that

selective expansion and/or targeting immigration of Foxp3+ Treg cells allowed effective therapy or retroconversion of several inflammatory/autoimmune-related disorders, including type 1 autoimmune diabetes, experimental colitis and experimental autoimmune enchephalomyelitis. It is reasonable to believe that Foxp3+ Treg cells could be a new target of OLP treatment (6).

MATHERIALS AND METHODS

Immunhistochemistry

Immunohistochemistry is the localization of antigens or proteins in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, or colloidal gold.

In this study multicolor immuno-flourescence was used to examine CD3, CD25, CD152 and Foxp3.

Matherials

Biopsis from 12 patients with clinical OLP or LR were included. However, histopathological analysis indicated that only seven of these twelwe biopsies were in accordance with OLP/ LR. The histopathological analysis indicated that one biopsy corresponded with chronic discoid lupus erythematosus, two were histopathologically diagnosed leukoplakia and two were not given any diagnosis at all, only concluded not to be in accordance with OLP/ LR.

Methods

Cell quantification:

CD3+ T cells were examnined with a zeiss Axioplan2 microscope at 400 x magnification. The number of intraepitelial cells was sometimes so low that quantification including these would not give at reliable result, it is therefore only the cells in the subepithelial infiltrate that are included in the cell quantification in this study. The subepithelial lymphocyte density was sometimes so high that one could not discrimination individual cells. In such cases the results were taken from deeper part or in the edge of the infiltrate.

We were doing trippel labeling where median 418 CD3+ T cells (range 203-576) were identified and further examined for Foxp3 and CD25 expression. Median 222 CD3+ T cells (range 205-411) were identified and further examined for Foxp3 and CD152 expression.

RESULTS

Biopsies from 12 patients were examined based on the clinical tentative diagnosis OLP/LR. 7 had all of the histopathological criteria of OLP/LR. The remaining five had clinical signs and T cell dominated subepithelial band shaped infiltrate but the histopathological picture was not in accordance with OLP/LR. Based on histopathological examination made by a specialist in pathology, one was diagnosed chronic discoid lupus erythematosus, two leukoplakia and two remained uncertain. These remaining five are placed in a cathegory by them selves named ”other inflammatory disorders”-group. Table 1 shows an overview of the 7 biopsies that are included in the OLP/LR-group.

Biopsy arcive reference	**Diagnosis**	**Type**
LR 7,2	Lichen planus/ licheniod reaction	Moderate
LR 31	Lichen planus	Intense
LR 32	Lichen planus/ lichenoid reaction	Moderate to intense
LR 34	Lichen planus/ lichenoid reaction	Moderat to intense
LR 38	Lichen planus	Moderat til intens
LR 39	Lichenoid reaction	Intense
LR 40	Lichen planus	Intense

Table 1: *An overview of the seven OLP/LR biopsies.*

31% of the subepithelial T cells expressed Foxp3 and 55% were CD3+Foxp3+CD25+ Tregs

Trippel labelling for CD3, Foxp3 and CD25 revealed that median 31% (range 17% - 58%) of the CD3+ T cells in the oral lichen biopsies expressed nuclear Foxp3 (Figure 3). Furthermore, median 55% (range 15%-69%) of the Foxp3+ T cells were CD25+ Treg-cells **(Figure 4,** Figure 5**)**.

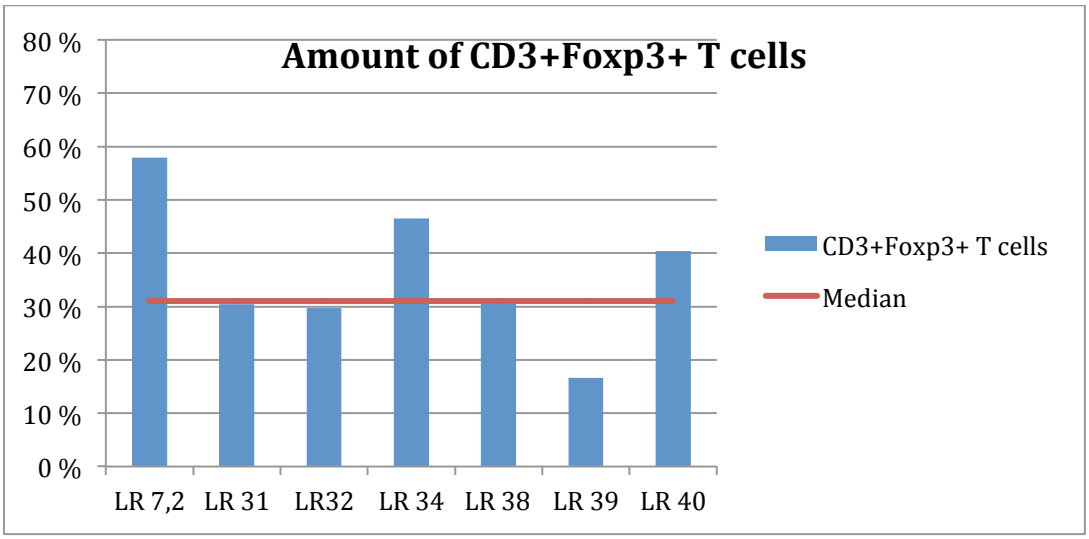

Figure 3: *CD3+ T cells with nuclear Foxp3 expression*

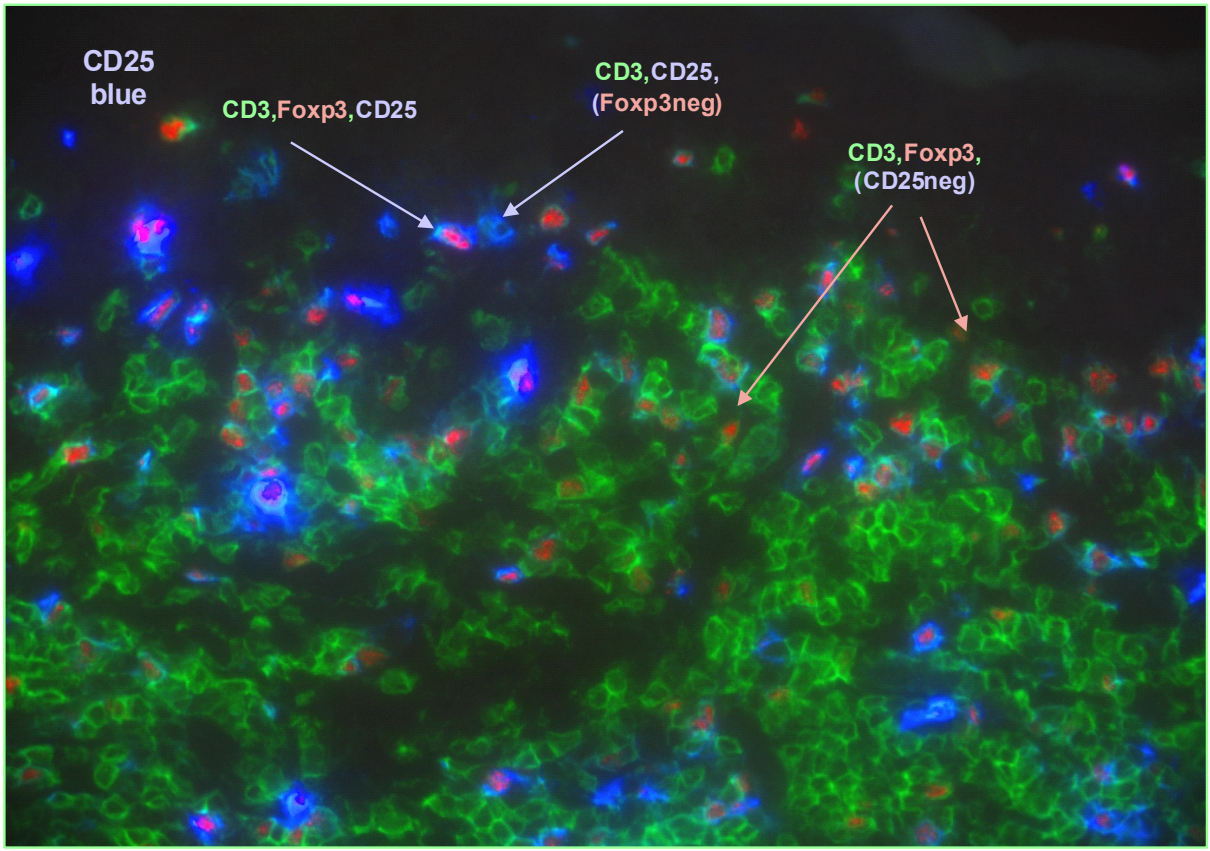

Figure 4: *Foxp3+ (red), CD25+ (blue) T cells (CD3, green) in oral lichen planus. Note both CD25+ Tregs, and CD25-negative Foxp3+ T cells can be seen in the subepithelial infiltrate (microphotograph from Koren et al, 2007) (12).*

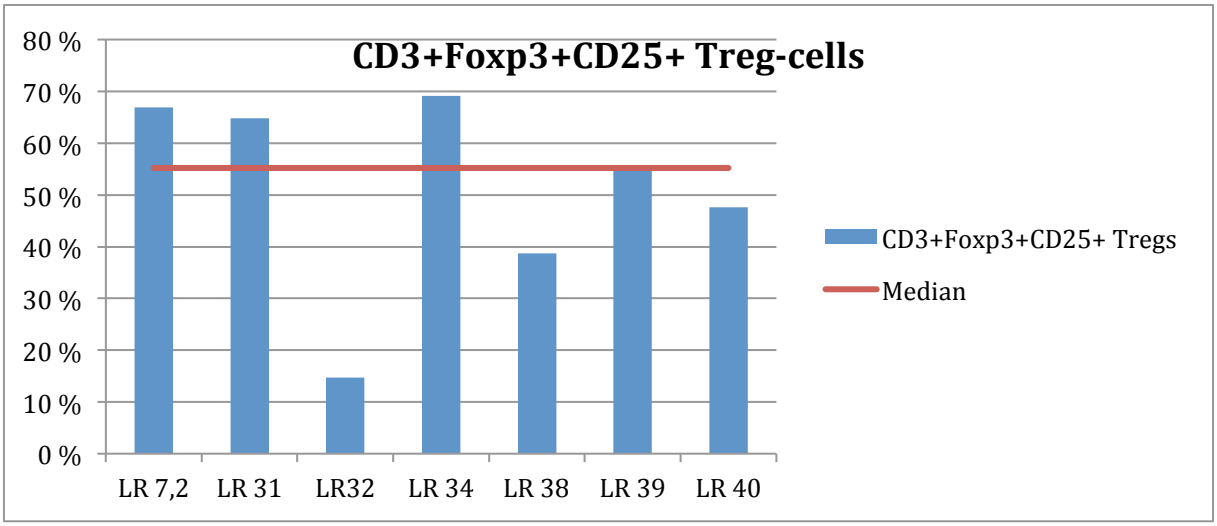

Figure 5: *Percentage of Foxp3+ T cells (CD3+) coexpressing CD25*

31% of the CD3+Foxp3+ cells coexpressed CD152+

Median 31% (range 5%-67%) of the CD3+Foxp3+ T cells were CD152+ Treg-cells **(**Figure 6**).**

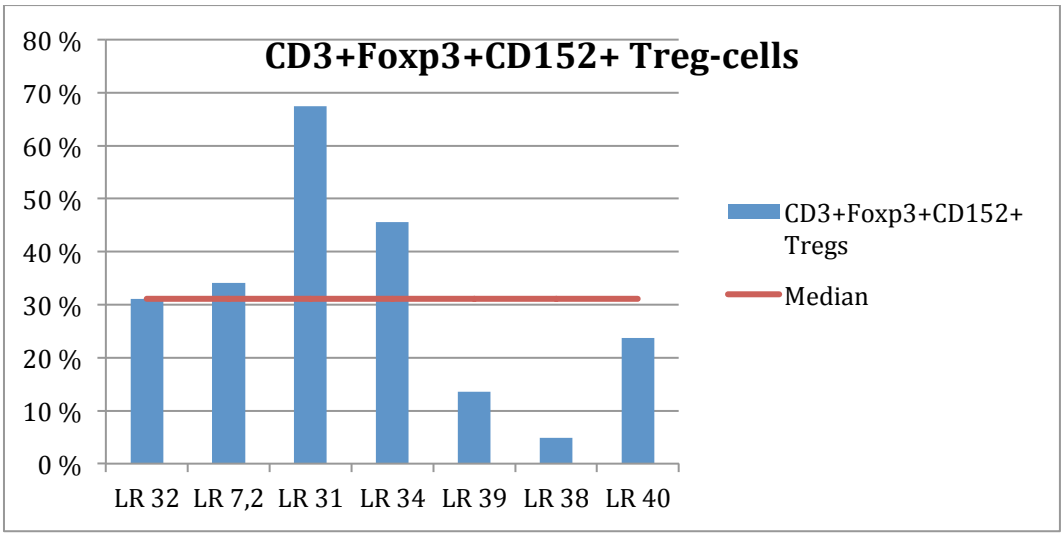

Figure 6: *Percentage of Foxp3+ T cells (CD3+) coexpressing CD152*

Minority of Tregs in the subepithelial ifiltrates

CD3+Foxp3+CD25+ Treg-cells were median 19% (range 9%-39%) of the total number of T cells in this counting **(Figure 7)**. The amount of CD3+Foxp3+CD152+ Treg cells

made only a small share of median 7% (range 1%-20%) of the total number of T cells counted **(**Figure 8**).**

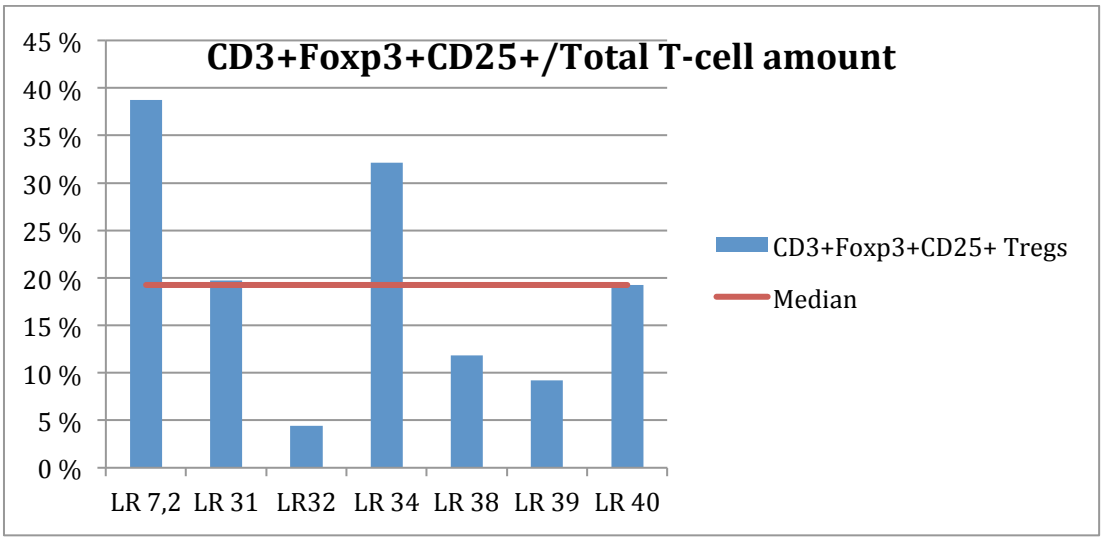

Figure 7: *Percentage CD3+Foxp3+CD25+ Treg-cells among the total number of T cells.*

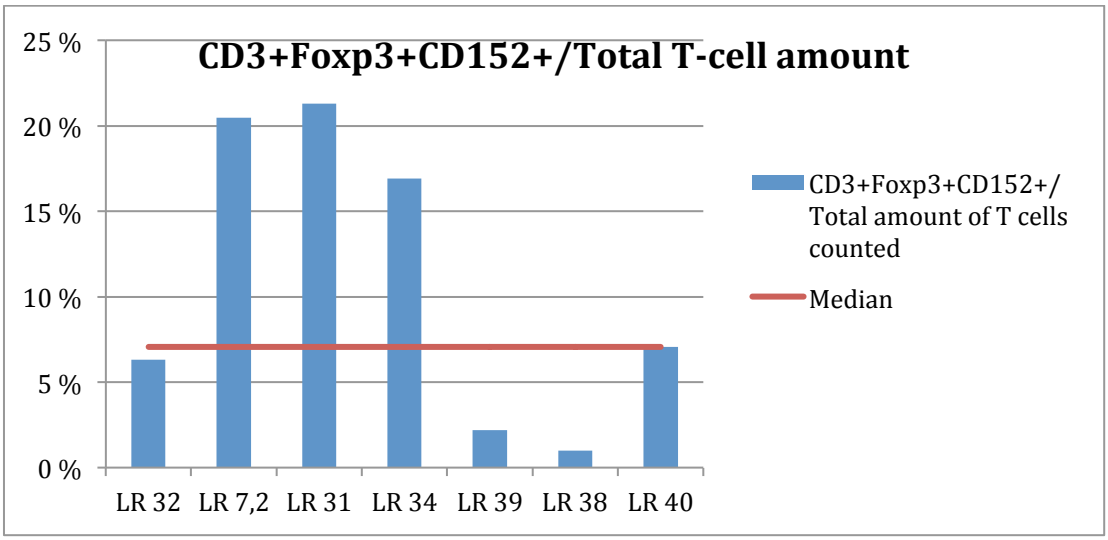

Figure 8*: Percentage CD3+Foxp3+CD152+ Treg-cells among the total number of T cells.*

Trippel labelling for CD25, Foxp3 and CD152

Five biopsies were given trippel labelling to reveal the expression and combinations of CD25, Foxp3 and CD152. Only two of these biopsies were diagnosed OLP/LR. Therefore, this result is based on cell counting from these two biopsies only. 42% (range 39%-45%) of all CD25+ cells were CD25+Foxp3+ Treg cells. 38% (range 25%-50%) of all

CD25+Foxp3+ regulatory T cells were positive for CD152 **(**Figure 9**).** Of all CD25+ T cells, an amount of median 16% (10%-23%) were CD25+Foxp3+CD152+ regulatory T cells **(Figure 10)**.

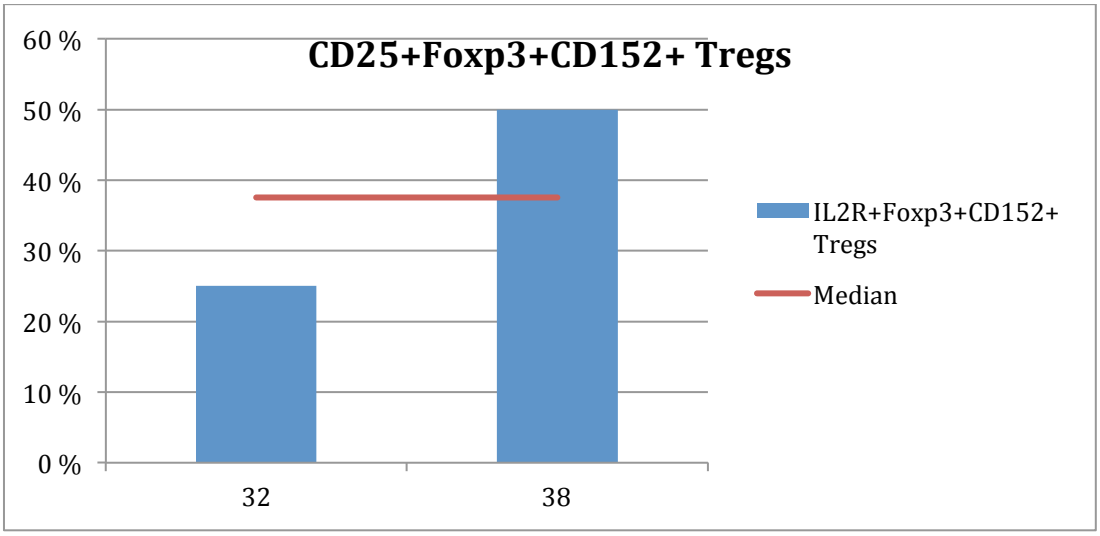

Figure 9: *CD25+Foxp3+CD152+ Tregs.*

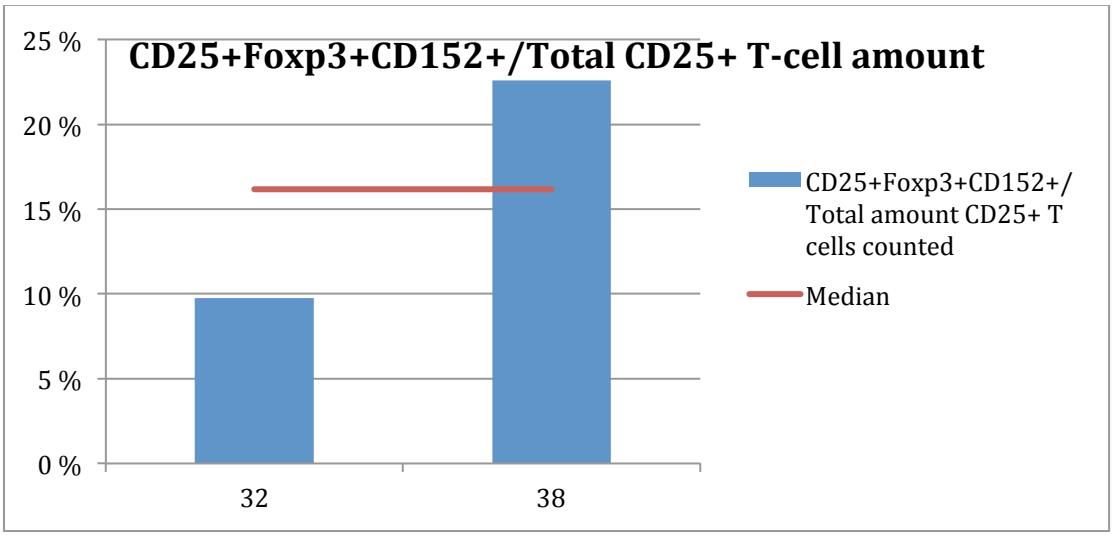

Figure 10: *Percentage CD25+Foxp3+CD152+ Tregs of total CD25+ T cells.*

The ”other inflammatory disorders”-group showed a higher lewel of CD3+Foxp3+ cells and a somewhat lower amount of these cells were CD152+ and CD25+

In table 2 below there is an owerview of the five biopsies that are included in this group and their diagnoses.

Biopsy arcive reference	Diagnosis
LR 29	Chronic Discoid Lupus Erythematosus
LR 30	Leukoplakia
LR 33	Leukoplakia
LR 36	Uncertain
LR 37	Uncertain

Table 2: *Overview of the five non OLP/LR biopsies*

Median 43% (range 30%-71%) of all CD3+ T cells were Foxp3+ T cells **(**Figure 11**)**.

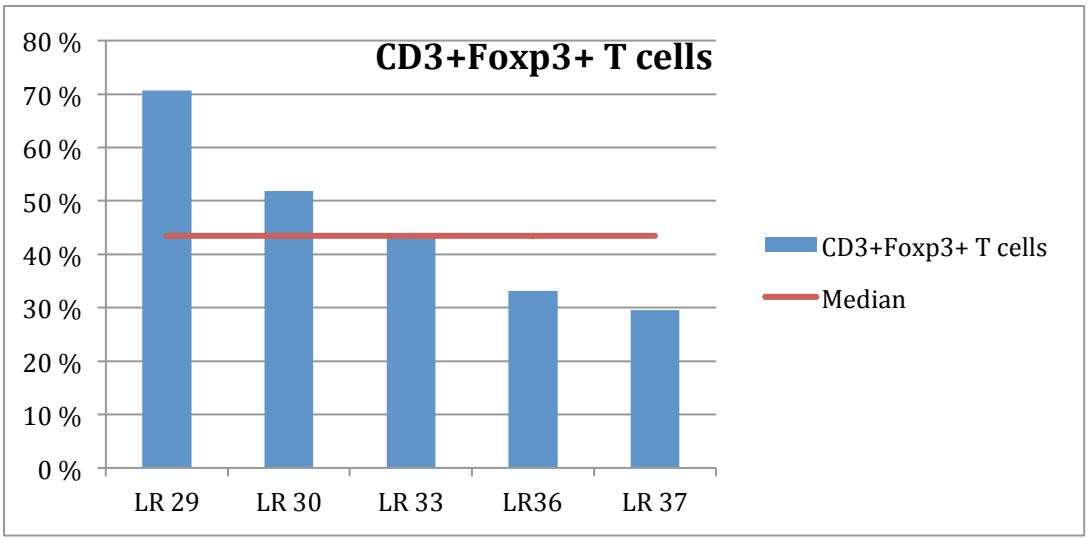

Figure 11: *Precentage of CD3+ T cells expressing Foxp3 in "the other lesions".*

Median 48% (range 27%-72%) of these Foxp3+ T cells coexpressed CD25 and were thus phenotypically Tregs **(Figure 12).**

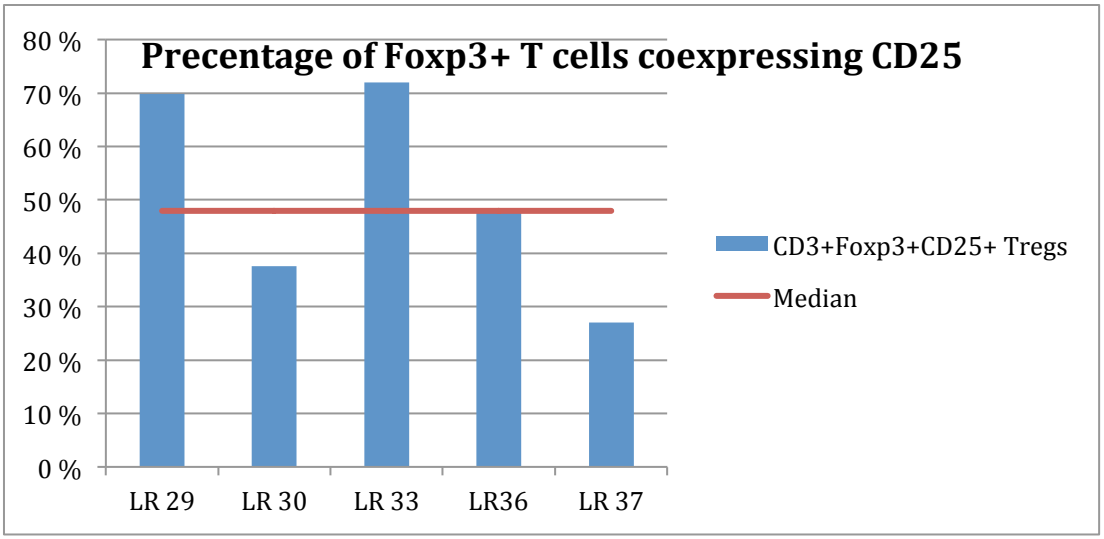

Figure 12: *Precentage of Foxp3+ T cells coexpressing CD25 in "the other lesions".*

Median 19 % (range 4%-47%) of the Foxp3+ T cells coexpressed CD152+ **(**Figure 13**).**

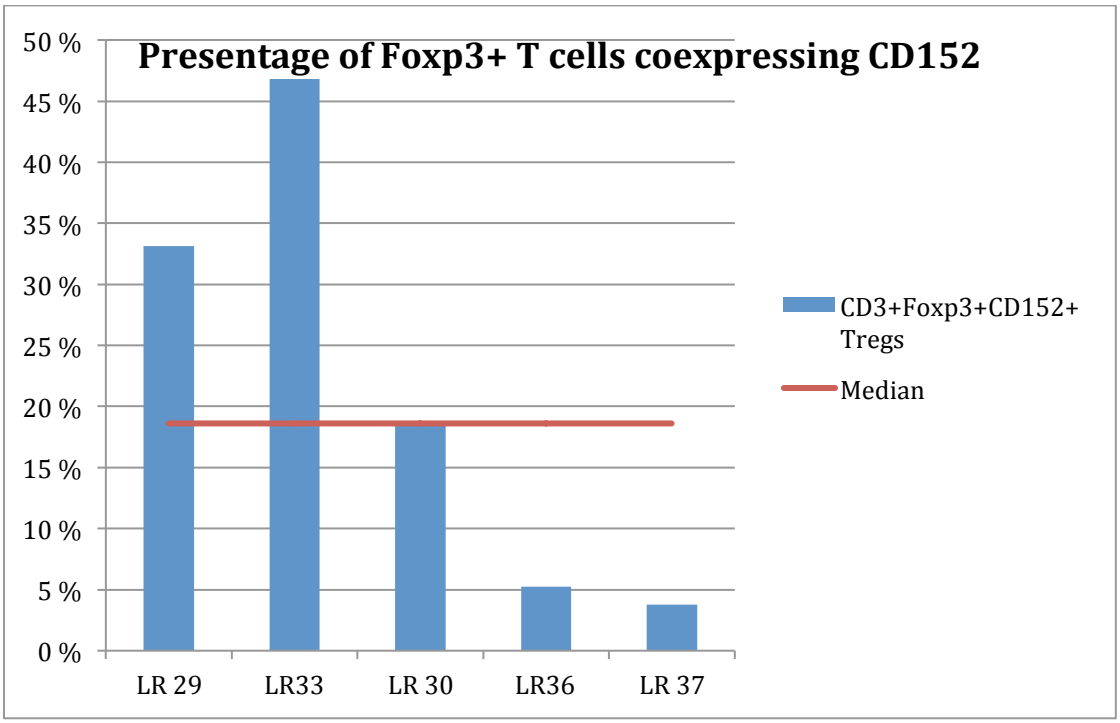

Figure 13: *Precentage CD3+Foxp3+ T cells coexpressing CD152 in "the other lesions" .*

Of the total amount CD3+ T cells counted median 19% (range 8%-49%) were CD3+Foxp3+CD25+ Treg cells **(**Figure 14**).**

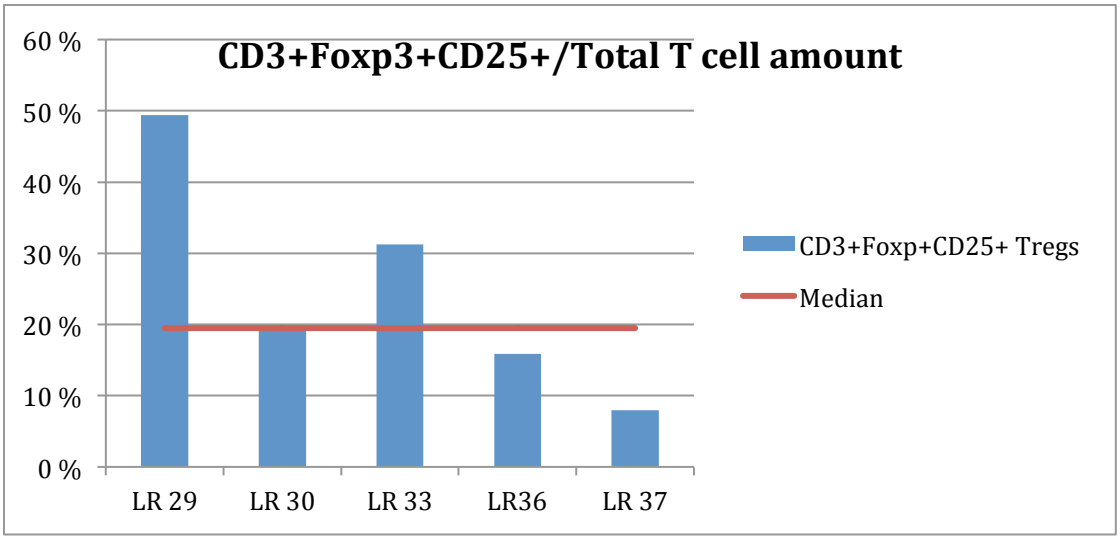

Figure 14: *Precentage of T cells (CD3+) coexpressing Foxp3 and CD25 in "the other lesions".*

Moreover, median 11% (range 1%-21%) of the T cells coexpressed both Foxp3 and CD152 **(**Feil! Finner ikke referansekilden.**Figure 15)**.

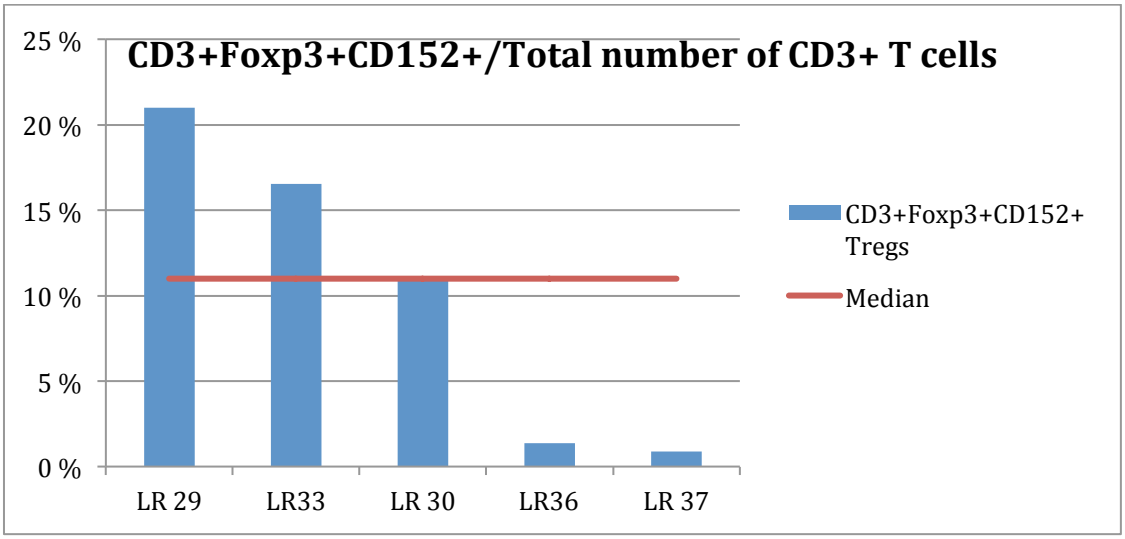

Figure 15: *Precentage of Foxp3+CD152+ T cells (CD3) of total CD3+ T cells in "the other lesions".*

DISCUSSION

This study is meant as a compliment to the work of Koren *et al.* on regulatory T cells in oral lichen planus. The aim of the study is to examine if inhibitory T cells are located in the subepithelial lymphocyte band in oral lichen. The crucial roles of CD4+CD25+Foxp3+ Treg cells have been identified in a series of autoimmune and/or inflammatory diseases. It is reasonable to believe that their role in the pathogenesis of OLP should not be overlooked, but rather be examined further (6). Research on these cells will guide us nearer to the goal in understanding the diseasemechanism in OLP and lead us closer to an ability to offer treatment on this area. By combining antibodies to CD3, Foxp3, CD25 and CD152 the phenotypical combinations of T cells with regulatory properties in the subepithelial infiltrate of OLP were identified.

In this study only a few CD25-stained cells were negative for Foxp3 staining. Most of the Foxp3+ cells co-expressed CD3. These findings are in accordance with those of X-a Tao *et al*, 2009; Rieger *et al*, 2006; de Boer *et al*, 2007. Median 30% of the subepithelial CD3+ T cells coexpressed Foxp3, compared with median 40% in the study of Koren *et al*. The majority of Foxp3+ cells (median 55%) also co-expressed CD25. In the study of Koren *et al* this number was as high as median 88% in OL and median 83% in controls. Consequently median 19% of the total amounts of CD3+ T cells in the subepithelial infiltrate in oral lichen biopsies were CD3+Foxp3+CD25+ immune regulatory T cells in this study. Koren *et al* found that median 33% of the T cells expressed a CD3+Foxp3+CD25+ regulatory phenotype in the subepithelial infiltrate of oral lichen and median 12% in the normal controls. Further, median 31% of the CD3+Foxp3+ T cells were CD152+ Treg-cells, with a wide range of 5%-67%. Koren *et al* found that median 83% of the subepithelial Foxp3+ T cells co-expressed CD152 in oral lichen, and 66% in controls. The amount of CD3+Foxp3+CD152+ Treg cells were only median 7 % of the total number of CD3+ T cells in this study.

The results here show consistently lower values than those from the study of Koren *et al*. One explanation for this may be that there in the biology always will be a noticable variation between individuals. These variations are more visible in a small collection of data, which is the case here with seven OLP/LR biopsies compared with Korens eleven biopsies. Neither can one ignore that bias may occure due to manual subjective cunting

and no independent observers. Another element that must be taken into concideration is that one share of the biopsies that are included in this study was fixated (biopsies with LR values from 33-40, **(table 1)(table 2)**), while the other share was not. In the study of Koren *et al* no biopsies were fixated. The results here show no noticable discrimination between values from fixated and not fixated biopsies, but the matter should still be mentioned. Although the exact numbers variate, we can se that the amount of CD3+Foxp3+CD25+ Tregs is upregulated in oral lichen biopsies in both studys comparing with the controls.

Resting Treg cells that are being isolated directly from noninflamed tissue have no, either suppressor or proinfalmmatory function. Only after activation they gain a meaningful level of Treg cell function. Simply counting Foxp3+ cells give no indication whether these are in an activated or a resting state (5). Also, Foxp3 may be present even though it`s inactive or possess abnormal function. There are several mechanisms that contributes to expression of Foxp3 even though it`s not in function. For Treg cells to maintain their suppressive function, Foxp3 demands co-operation with other transcriptional factors. Moreover, Foxp3+ Treg cells are not terminally differentiated cells, and can differentiate into a variety of Teff cells (5).

The exact trigger-stimuli for Treg cells are incompletely understood. It seems clear that the activation is driven by an antigen dependent reaction with APCs. The APCs are in turn driven by the local cytokin milieu. Antigens are, via APCs (e.g dendrittic cells) assumed to play a critical role in the activation of Treg cells. These antigens may be self antigens or antigens from the mucosal surface. Certain inflammatory conditions and tumors are also believed to trigger Treg cell activation. The latter two are in turn involving antigens. The exact role of the local inflammation is still uncertain when it comes to activation of Treg cells, but it probably displays an important role, both in activation of local APCs, and when it comes to guiding the local cytokin milieu. Based on a variety of studys, cytokines such as TGF-beta, IL-10 and IL-2 are of importance for Treg activaion and/or maintainance of suppressor functions. nTreg and iTreg cells differentiate in the thymus and periphery, respectivly. The differentiation of Th17 and iTreg from naive T cells depends on the level of TGF-beta expression. High levels of TGF-beta in the tissue microenvironment may lead to the developement of iTreg by signalling

through STAT5. This results in the upregulation of Foxp3. Also, Treg cells can develope from thymic CD4+ T cell precursors in the presece of TGF-beta and IL-2. These are termed natural Treg cells (4). Loss of the balance between Th17 and Treg cells will break immune homeostasis in the host and lead to the developement of autoimmune diseases. (5). Interactions between CD152, GITR, PD-1 and PD-1 ligand on Treg cells and the respective surface ligands on other celltypes (e.g APCs) may also be of importance for Treg cell activation. Dendrittic cells that express the immunoruppresive enzyme IDO are directly able to activate mature Tregs. Local conditions that induce dendrittic cells to express IDO, such as tumors, TLR-ligands and infections may activate Treg cells in an IDO-dependent manner.

Certain markers caracterize activated or effector memory phenotype in Tregs. Tregs are defined by CD25, CD39, Foxp3, CD152 and GITR (8). These markers identify populations or subgroups of Tregs, but are not validated as unambiguous markers of the activated functional condition. For instance, foxp3 can be co-expressed and can interact (4) and most surface proteins that are expressed on CD4+CD25+ Treg cells such as CD25, CD152, GITR and PD-L1 can also be found on activated T responder cells (6). The suppressive activity is still based on in vitro measurements.

Treg pasticity is a term describing Treg cells that express cytokins normally associated with helper/effector CD4+ T cell phenotypes. Suppression is clearly the most dominant function of Treg cells, but several studies indicate that these cells also play a crucial role as T-helper cells. Foxp3+ Treg cells can under certain local physiologic conditions be induced to express IL17, IFN-gamma and IL-2. These are cytocines typically associated with helper/effector CD4+ T cell phenotypes. There are several published results illustrating that Treg cells are losing their Foxp3 expression when converted to proinflammatory Th cells. Peripheral Tregs are a heterogenous group of cells that include some cells with transient or unstable Foxp3. Either these can be Treg cells that are newly converted in the periphery and that retain the possibility to reverse to effector cells, or they can be cells that are under normal differentiation into Th17-cells and then express Foxp3 in an early stage. No one of the two latter represent autentic, fully formed Treg cells, so their reversion into a proinflammatory phenotype will not involve reprogramming. Studys have confirmed that cytokines, such as IL-6, of the

congential immune system can drive Treg reprogramming. Similar effects are reported for the proinflammatory enzyme IL-1beta. Based on the fact that Foxp3 may be present, but not in function, transient or unstable, the definition of reprogrammed Treg cells ought to be functional, rather than defined by loss of Foxp3.

Despite a positive expression for both CD3 and Foxp3, a high amount of cells are negative for CD25 and CD152 (45% and 69% respectively) in this study. These may be cells with inactive Foxp3 that do not possess a T-regulatory function. On the other hand, these numbers are higher than those described in the study of Koren *et al*. which indicate that there may be other, perhaps technical explanations.

The ”other inflammatory disorders” – group consists of biopsies from five patients that histopathologically not were in accordance with OLP/LR. The immunohistichemical analysis show some interesting results here compared with results from the oral lichen panus/LR-group that are described above. First, there is a higher amount of Foxp3-expression among CD3+ cells in this group, and the expression of CD25 and CD152 does not variate significantly. Further, 19 % of the total cell number were CD3+Foxp3+CD25+ and 11% CD3+Foxp3+CD152+ compared with 19% and 7% in the OLP/LR respectivly.

The five biopsies in the ”other inflammatory disorders”-group had clinical signs of OLP/LR and a T cell dominated subepithelial band shaped infiltrate. Even so, the histopathological picture did not correlate with OLP/LR. One was diagnosed chronic discoid lupus erythematosus, two leukoplakias and two remained uncertain. In recent years, abnormalities in number and function of Foxp3-expressing CD4+CD25+ Treg cells have been identified in several inflammatory diseases, including psoriasis, multiple sclerosis, autoimmune polyglandular syndrome type II, rheumatoid artritis, myastenia gravis and type I diabetes (6). Results from the ”other” group here indicate that more inflammatory diagnosis should be added to this list and that Foxp3+ Treg cells could be a new target of OLP treatment, as well as of treatment of a number of other inflammatory diseases. Many studies have shown that selective expansion and/or targeting immigration of Foxp3+ Treg cells allowed effective therapy or retroconvention of several inflammatory/autoimmune-related disorders (6).

Another approach may be to question the diagnostic criteria when both a clinical picture that correlates, a histopatological picture that partly correlates and histopathology with no significant variation from oral lihen planus/LR.

Through this study I have acquired experience in immunohistochemical techniques and the project has been a big learning experience for me. By increasing the number of patient biopsies and by using an independent observer the results would have been even more reliable, but there were either enough patients available or more time to spend. Therefore, this is it for now.

CONCLUSION

This study has demonstraded the prescence of Treg cells in OLP/LR. The results are compared with, and meant as a compliment to the the study of Koren *et al*. Even if the results not completely correlate, both studies demonstrate an increased number of Treg cells compared with a control-group. In addition, we have immunhistochemical analysis of a group of bipsies from inflammatory lesions not diagnosed OLP/LR, and these results are strikingly correlated with results form the OLP/LR-group on Treg expression. This may raise a question about the diagnostic criteria of OLP, but may also demonstrate that Treg cells may be an important target when it comes to treatment of other inflammatory disorders as well. The two latter issues may be fundament for future studies.

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